

Sensitivity of Lymphocytes from Vulcanizers to the *in Vitro* Induction of Sister Chromatid Exchanges

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Spontaneous frequencies of sister chromatid exchanges (SCEs) and SCEs induced *in vitro* by chemicals with different mechanisms of action such as mytomyecin C, 4-nitroquinoline oxide, and 3-aminobenzamide were examined in phytohemagglutinin-stimulated peripheral blood lymphocytes from a group of workers in a rubber plant and a control group, both of which had been analyzed for levels of spontaneous SCEs 2 years earlier. An interindividual variability in the induction of SCEs was found after *in vitro* treatments with the different mutagens, which did not correlate with occupational exposure. This variability in the sensitivity to the induction of SCEs might be correlated to genetic differences among individuals, which have to be taken into account in environmental monitoring programs.

Introduction

The level of chromosomal aberrations in long-lived circulating lymphocytes is considered a reliable biological dosimeter of the absorbed radiation dose (1). With chemical mutagens the situation is more complex because the frequencies of chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) may be affected by interindividual differences in metabolism and susceptibility to chemicals. However, cytogenetic tests on chemically exposed populations have been able to detect different genotoxic exposures (2-4).

In previous work (5), the frequencies of chromosomal aberrations and SCEs in peripheral blood lymphocytes of vulcanizers working in the rubber industry were studied. This is a typical case where human biomonitoring may be justified, considering the organization of the working process and the complex interactions among the several hundred chemicals used, which may result in the formation of new carcinogenic materials, making it difficult to search

for specific carcinogenic agents. Known genotoxic agents such as benzene, benzo[a]pyrene, and nitrosamines have been detected in the effluents from rubber vulcanization (6), and epidemiological studies have shown that cancer risks still exist in the rubber industry (6).

No differences were found in our investigation between 34 vulcanizers and 16 control individuals for CAs or SCEs. Cigarette smoking was clearly associated with increased frequencies of SCEs in both exposed workers and controls. In a parallel study, Benigni et al. (7) estimated DNA repair ability in the same population by measuring *in vitro* UV-induced unscheduled DNA synthesis (UDS) in peripheral blood lymphocytes. In spite of the considerable interindividual variability, decreased UDS values were observed among vulcanizers compared to controls.

Therefore, in spite of the lack of detectable cytogenetic alterations and the uncertainties concerning the correlation between DNA excision repair and the genetic endpoints studied, it could be argued that vulcanizers could be at higher risk for mutagenesis and carcinogenesis.

Two years later, we further investigated a smaller group of the same vulcanizers and controls for both UDS and SCEs. In this second survey, the UDS was not different between the two populations, which may be explained by the high interindividual variability in the UV-induced UDS response.

Blood samples of vulcanizers and controls were exposed *in vitro* to known mutagens with different modes of action to detect whether the *in vivo* exposure to chemical agents even at levels that did not produce cytogenetic effects could

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modify the sensitivity of lymphocytes to other mutagenic agents and to verify whether vulcanizers had lower DNA repair ability. Mitomycin C (MMC) was chosen as a direct-acting crosslinking agent; 4-nitroquinoline oxide (4-NQO) was used because it mimics effects of ultraviolet radiation and may be useful to detect UV sensitive individuals. 3-Aminobenzamide (3-AB) is known to increase the spontaneous frequency of SCEs when present during the S-phase of the cell cycle. It is a very potent inhibitor of poly(ADP-ribose)polymerase (8).

Materials and Methods

Exposed Individuals and Controls

Blood samples were obtained from eight vulcanizers and seven employees of a rubber plant in central Italy. Working conditions and selection of the subjects were presented in a previous paper (5). Table 1 shows the demographic data used for statistical correlations.

Sister Chromatid Exchanges

From each blood sample, eight cultures were set up using 0.5 mL whole blood in 5 mL Ham's F-10 medium (Flow, Milano, Italy) with 20% fetal calf serum (Flow), antibiotics, glutamine, 0.02 mg/mL phytohemagglutinin (Wellcome, Pomezia, Italy) and 9 µg/mL 5-bromodeoxyuridine (BdUR; Sigma, St. Louis, MO USA).

Duplicate cultures were exposed 24 hr after stimulation to 0.009 µM MMC (Kyowa, Tokyo, Japan), 1 µM 4-NQO (Sigma), or 5 mM 3-AB (Sigma) until harvesting (51 hr later). Colchicine at the final concentration of 5×10^{-7} M

was added 71 hr after PHA addition, and cultures were fixed 3 hr later. Chromosome preparations were produced using the air-drying technique. Slides were differentially stained as previously described (9).

For each experimental point, 100 cells were scored for M_1 , M_2 , M_3 ratio to calculate the proliferation rate index (PRI) as follows: $PRI = (M_1 + 2 \times M_2 + 3 \times M_3) / \text{total metaphases scored}$. Forty second-division metaphases were scored for SCEs.

Results and Discussion

Figure 1 and Table 2 show that no differences in the yields of SCEs were found in the two repeated measurements between vulcanizers and controls. Therefore, the frequencies of spontaneous SCEs in 1982 and 1984 in the total number of individuals are also reported in Table 2. The lower frequency of SCEs observed in the whole population 2 years later could be ascribed to technical differences in culture conditions in the second examination as confirmed by the higher proliferation rate index shown in Table 3.

Table 2 shows the induction of SCEs by different mutagenic agents. The data show that there are no differences in the induction of SCE by the three chemicals between vulcanizers and controls. PRI values following MMC,

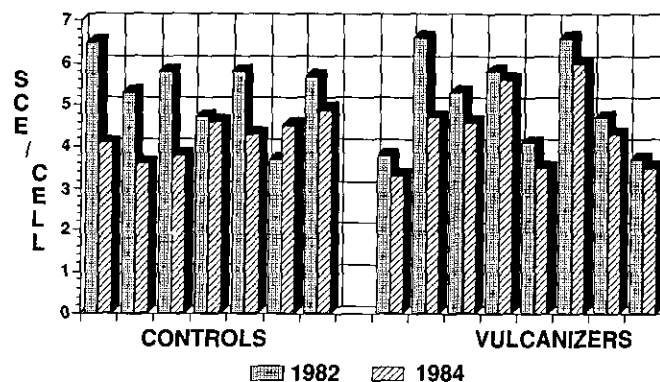


FIGURE 1. Spontaneous frequencies of sister chromatid exchanges in two repeated measurements in controls and vulcanizers.

Table 1. Main characteristics of the population.

	No. of Individuals	Smokers	Age, years	Exposure, years
1982				
Controls	16	8	39.9	—
Vulcanizers	34	20	39.1	8.2
1984				
Controls	7	4	43.7	—
Vulcanizers	8	5	40	11.2

Table 2. Average (\pm SD) spontaneous SCEs and SCEs induced by different chemicals in controls and vulcanizers.

	Spontaneous 1982	Spontaneous 1984	MMC	4-NQO	3-AB
Controls	5.3 ± 0.9	4.2 ± 0.4	35.3 ± 6.0	9.0 ± 0.8	8.3 ± 1.6
Vulcanizers	5.0 ± 1.1	4.4 ± 1.0	35.5 ± 6.5	9.6 ± 1.9	9.2 ± 1.0
Total	5.2 ± 1.0	4.3 ± 0.7	35.4 ± 6.0	9.3 ± 1.5	8.8 ± 1.4

Abbreviations: SCE, sister chromatid exchange; MMC, mitomycin C; 4-NQO, 4-nitroquinoline oxide; 3-AB, 3-aminobenzamide.

Table 3. Proliferation rate indexes in controls and vulcanizers.

	1982	1984	MMC	4-NQO	3-AB
Controls	1.22 ± 0.10	1.98 ± 0.27	1.48 ± 0.18	1.65 ± 0.26	1.84 ± 0.20
Vulcanizers	1.19 ± 0.13	1.98 ± 0.34	1.55 ± 0.11	1.93 ± 0.27	1.92 ± 0.31
Total	1.20 ± 0.17	1.98 ± 0.29	1.51 ± 0.15	1.78 ± 0.29	1.88 ± 0.25

Abbreviations: MMC, mitomycin C; 4-NQO, 4-nitroquinoline oxide; 3-AB, 3-aminobenzamide.

4-NQO, and 3-AB are shown in Table 3. A clear reduction in PRI is observed only after MMC treatments. The same reduction is observed in controls and vulcanizers, indicating that similar cell populations were analyzed. These results indicate that in our experiments the *in vivo* exposure of vulcanizers to low doses of genotoxic chemicals do

not affect the response of their lymphocytes to subsequent *in vitro* treatments with known mutagens.

Figure 2 presents the values of SCEs found after the *in vitro* treatments with different chemicals in each individual. The data show that different individuals have different susceptibilities to various chemicals and that the

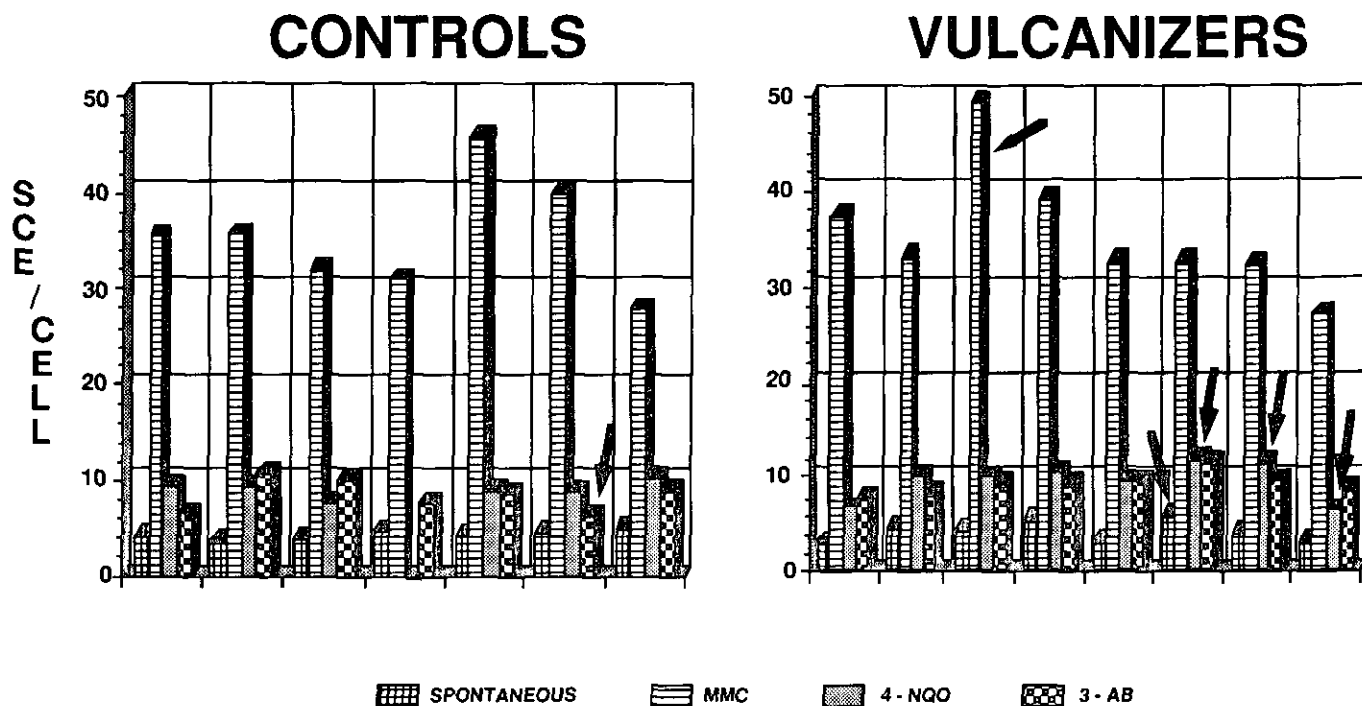


FIGURE 2. Individual values of spontaneous frequencies of sister chromatid exchanges (SCEs) and SCEs induced by different chemicals in seven controls and eight vulcanizers. Arrows indicate individuals exceeding 2 SDs in one treatment.

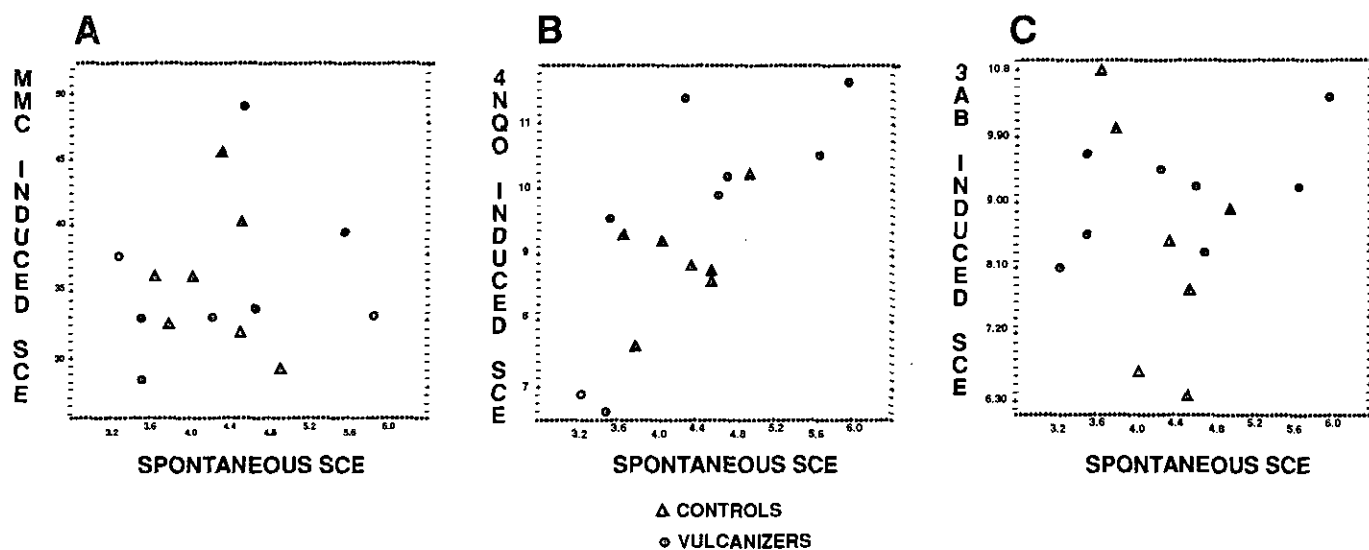


FIGURE 3. Correlations between spontaneous frequencies of sister chromatid exchanges (SCEs) and SCEs induced by mitomycin C (A), 4-nitroquinoline oxide (B), 3-aminobenzamide (C) in controls and vulcanizers. A pair of SCE values (spontaneous and induced) were plotted as a point for each individual.

sensitivity in the induction of SCEs in the same individual depends on the chemical. Individuals exceeding two standard deviations are shown in Figure 2. As can be seen from Figure 2, interindividual variability was present both in vulcanizers and in controls. This result indicates that the variability in response to *in vitro* mutagenic treatment is not correlated to occupational exposure but is more characteristic of the individual and the chemical used for treatment.

To verify whether individuals with low spontaneous yields of SCEs are less sensitive to mutagen-induced SCEs, we have graphically correlated the two variables as shown in Figure 3. No correlations were found, suggesting that the response in SCEs after mutagen treatment is not directly related to the spontaneous levels of SCEs. This finding of differences in the frequencies of SCEs among individuals following *in vitro* exposure to mutagens has also been found by other authors (10-12) and might be related more to genetic differences among individuals than to other factors (lifestyle, workplace, smoking, etc.). These observations have to be taken into account in environmental biomonitoring programs.

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